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There is evidence suggesting that life-style factors play a significant role in breast cancer (BC) development. Insulin, which secretion is influenced by life-style factors, might represent the possible etiological linkage between lifestyle characteristics and BC. Insulin might act in the carcinogenetic process through mitogenic effect on breast epithelium. Furthermore, insulin modulates an additional proliferative and hormone-regulating factor which may be involved in the etiology of BC: the Insulin-like Growth Factor I (IGF-I).

The investigators have proposed to test the hypothesis of the linkage between serum insulin and BC in an Italian prospective cohort study conducted to investigate the role of hormones and diet in the etiology of BC (the ORDET study). 10,788 healthy volunteer women, aged 35-69, residents in Varese province, an area covered by the Lombardy Cancer Registry, were enrolled between June 1987 and June 1992. At the recruitment, blood samples were collected between 8:00 and 9:30 A.M., after an overnight fast, and stored at -80° C. During the first seven years of follow-up, the cancer registry identified 144 breast cancer cases. The study compares pre-diagnostic serum levels of insulin, IGF-I, free IGF-I, and IGF-I binding proteins (IGFBP-I and IGFBP-3) for the BC cases and 576 controls (four per BC case) randomly selected from the cohort women who did not develop BC during same follow-up period, matched on age, menopausal status, recruitment center, and recruitment period.

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Bde Li 6/25/01

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Introduction

The primary purpose of this study is to examine insulin and insulin-growth-factor pattern in relation to breast cancer etiology. In addition, it also evaluates the possible concomitant role of sex steroids in the causal relation with breast cancer.

In this prospective cohort study, 10,788 healthy volunteer women, aged 35-69, residents in Varese province, an area covered by the Lombardy Cancer Registry, were enrolled between June 1987 and June 1992. At the recruitment, blood samples were collected between 8:00 and 9:30 A.M., after an overnight fast, and stored at -80° C. During the first seven years of follow-up, the cancer registry identified 144 breast cancer cases. The proposed study will compare pre-diagnostic serum levels of insulin, IGF-I, free IGF-I, and IGF-I binding proteins (IGFBP-I and IGFBP-3) for the BC cases and 576 controls (four per BC case) randomly selected from the cohort women who did not develop BC during same follow-up period, matched on age, menopausal status, recruitment center, freezer and position within the freezer, recruitment period, and recruitment within the same daylight saving period. The proposed study represents the first attempt to evaluate the association of insulin and IGF-I with BC using data from a cohort prospective study in which several potential sources of hormone variability have been controlled for by study design.

Insulin may play an important role in the etiology of breast cancer. Insulin, in fact, is a powerful mitogenic agent inducing a dose-dependent growth response in BC cell lines acting via its own receptor. In addition, insulin may play a role in tumor promotion by upregulation of ovarian steroid secretion. In fact, chronic hyperinsulinemia has been implicated in the etiopathogenesis of hyperandrogenic status and hypothesized as a determinant of this hormonal pattern. Furthermore, insulin may act as a tumor promoter through its effect on insulin-like growth factor-I (IGF-I): IGF-I is a structural homologue of insulin, characterized by both mitogenic, and gonadotropic action of its own.

In spite of the very strong physiological evidence for a role of insulin in BC etiology, very limited evidence has been presented in epidemiological studies. To date, only two case-control studies have been conducted on serum insulin and BC risk and the results of both studies supported the association. IGF-I has also been associated with breast cancer in several case-control studies. However, these case-control studies were relatively small, blood was collected in a non-fasting state, and there was no control of other potential sources of hormone variability (i.e., circadian rhythm). The only cohort study on this question showed a seven fold elevation in BC risk between the highest and the lowest tertile of IGF-I among premenopausal women. However, this study evaluated only total IGF-I serum levels, lacking description of other potential hormone/metabolic determinants of the disease.

Body Of Report

During the second budget year, we assayed the 720 samples (144 samples from breast cancer cases and 576 from control women), for Insulin, and glucose. For Insulin-Like-Growth-Factor 1, Free Insulin-Like-Growth-Factor, Insulin-Like-Growth-Factor Binding Globulin 1, Insulin-Like-Growth-Factor Binding Globulin 2, Insulin-Like-Growth-Factor Binding Globulin 3 we have completed an investigative phase to assess the most accurate assay method to use for population based studies. Several immunoassays have been developed to measure total IGF-I in serum or plasma. Hitherto, no autoantibodies have been raised which detect IGF-I bound to IGFBPs in the binary or ternary complexes. Therefore, in our study we identified the need to dissociate and separate IGF-I from IGFBPs before determination by radioimmunoassay using acid ethanol extraction, which is the most common technique, applied. Thus, we have now started and completed the extraction of IGF-I and its binding proteins 1, 2, 3 for more than half of the study subjects.

As a consequence, in the present annual report, we include the preliminary but complete findings of insulin and glucose in relation to breast cancer risk. The findings are here reported in the appendix section as an abstract accepted as oral communication at the Annual Meeting of the American Association Cancer Research in New Orleans, April 2001 and at the Annual Meeting of the Society for Epidemiological Research (Toronto, June 2001).

Publications

At the present time, there are no results or publications coming directly from this grant because we have completed only part of the analytical determinations. However, Dr. Muti has completed an additional study on breast cancer conducted within the ORDET cohort. The study concerns blood red cell fatty acid composition and risk of breast cancer. Since, variations in fat consumption and metabolism are suspected to contribute to the marked regional differences in breast cancer incidence rates, the fatty acid composition of the erythrocyte membrane may be an appropriate biomarker for investigating the relation of dietary fat and patterns of fatty acid metabolism to breast cancer. The association between prediagnostic red cell membrane fatty acid and post-menopausal breast cancer risk was analyzed among postmenopausal breast cancer cases and controls members of the ORDET study. Red cell membranes were separated and membrane phospholipids fatty acids were measured as a percentage of total fatty acid. Conditional logistic regression analysis was performed to test the association between the membrane's fatty acid composition and breast-cancer risk. The Saturation Index (SI), a ratio between membrane stearic and oleic that depends on the activity of the enzyme delta9-CoA (Δ9-d) desaturase has been also tested. The study results showed positive associations with breast-cancer risk were detected for oleic (OR=2.72; CI=1.26-5.91) and monounsaturated fatty acids (OR=3.9; CI=1.66-9.18). SI was

inversely associated to breast-cancer risk (OR=0.40; CI= 0.19-0.85) as well as linoleic acid (OR=0.42; CI=0.19-0.94). No significant association was detected with saturated fatty acids or with n-3 PUFA. Although the relative importance of diet in regulating the fatty acid composition of membranes remains to be fully determined, study results strongly indicate that oleic and monounsaturated fat components of the red blood cells are relevant predictors of breast cancer. The observed inverse association between breast cancer and membrane saturation index suggests the need of further studies connecting the pattern of its multiple determinants (dietary, metabolic, hormonal) to the development of breast cancer. Results from that study are in publication in the Journal of National Cancer Institute (see references Pala V.et al., JNCI).

Dr. Muti also completed a study on measurement variability of plasma β-sitosterol and campesterol during the past academic year. Phytosterols are plant sterols, which are structurally similar to cholesterol and characterized by anti-carcinogenic and anti-atherogenic properties. β-Sitosterol and campesterol are the predominant phytosterols in blood. The study was aimed to analyze reproducibility and overtime reliability of plasma β-sitosterol and campesterol measurements. In order to study reproducibility of the measurement (technical variability), three healthy premenopausal women donated a sample of their blood. Each blood sample was subdivided into six aliquots and analyzed within the same run by the same laboratory technician. The intraclass correlation coefficients (*ICCs*) of the assay for plasma β-sitosterol and campesterol were

0.88 and 0.94 (95% Confidence Interval low bounds [95% CI_{low}] were 0.66 and 0.82), respectively. To study reliability of β-sitosterol and campesterol measurement over time, seven premenopausal women were recruited. Over a six-month period, each woman provided once a month a fasting blood sample at the same time of day, and the same numerical day of luteal phase of menstrual cycle (between the 20^{th} and the 24^{th} day of the menstrual cycle). All plasma samples from the same individual were processed together at the same time by the same technician at the end of the six-month period. The overtime *ICC*s of plasma β-sitosterol and campesterol were 0.91 (95% CI_{low} 0.49) and 0.58 (95% CI_{low} 0.31), respectively. The high reproducibility and the good overtime reliability of plasma β-sitosterol and campesterol measurements indicate that they may be suitable for potential clinical and population based studies on cancer prevention. Mrs. Jianhong Li, a student working with Dr. Muti, used that study as her master thesis (see reference Li JH, et a., 2001)

In the past year Dr. Muti has collaborate in a study on methylation and breast cancer risk with Dr. Jo Freudenheim which resulted in an additional presentation to the Annual Meeting of the American Association Cancer Research in New Orleans (2001) and in a manuscript in preparation (see references for the abstract presentation).

In addition, she participated in the analysis of the effects of Luteinazing Hormone and Follicular Stimulating Hormone on the risk of breast

cancer. The study was conducted on the same groups of premenopausal women (breast cancer cases and controls) members of the ORDET cohort study. Results showed that the two protein hormones have protective effect on breast cancer. The data have been presented at the international meeting of the population based cancer registries of Latin countries (ORDET cohort is followed up by the Lombardy Cancer Registry) in Neuchâtel, Switzerland, Spring 2001, and at the annual meeting of the Italian Cancer Registries, Alghero, Italy, Spring 2001 (see references for the abstract presentations).

Dr. Muti has also conducted a study based on a DOD funded case-control study and in collaboration with the Department of Microbiology at the Johns Hopkins University, MD on the influence of human papillomavirus (HPV) on prostate cancer risk. Data showed that the prevalence of HPV infection was higher in prostate cancer cases than in controls. Data were presented by Dr. Womak, a NCI postdoctoral fellow working with Dr. Muti, at the Professional Development Workshop, sponsored by the Comprehensive Minority Biomedical Branch (CMBB), Office of Deputy Director for Extramural Science (ODDES), at the National Cancer Institute, June 2001 (see references for the abstract presentation).

Dr, Muti has also in publication studies regarding indices of oxidative stress and risk of chronic diseases. The studies were conducted using a dataset

developed at the Department of Social and Preventive Medicine with the collaboration of five different population-based studies (see references).

Key Research Accomplishments

- Biochemical analyses completed (Insulin, glucose)
- Statistical analysis performed for insulin and glucose
- Biochemical analyses for IGF-I, IGFPB-1, 2, 3 completed for more than half of the study members

Reportable Outcomes

Glucose metabolism showed to be involved in breast cancer development in premenopausal but not in postmenopausal women.

Conclusions

Analytical determinations of IGF-I, IGFPB-1, 2, 3 are underway. This information will further clarify the role of glucose and its metabolism in breast cancer etiology.

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Appendices

***Muti P**, Quattrin T, Misciagna G, Krog V, Micheli A, Browne R, Berrino F. Fasting serum glucose and insulin and breast cancer risk in premenopausal and postmenopausal women: the ORDET study. American Association Cancer Research, New Orleans, LA, USA, March 2001 [SER, Toronto, 2001].*

Fasting Glucose and Insulin: A Prospective Study

There is some evidence that glucose and other factors related to glucose metabolism, such as insulin may contribute to breast cancer development.
Objective To analyze the hypothesis that serum glucose, insulin levels are associated with breast cancer.
Design, setting and participants A nested case-control study design within a prospective cohort was conducted. From 1987 and 1992, 10,786 women aged 35-69 were recruited in a prospective study in Italy. At recruitment, blood samples were collected after 12 hours fast between 7:30 and 9:00 AM from all study participants. After 5.5 years, 144 breast cancer cases were identified among the participants of the cohort. Four matched controls were chosen for each breast cancer case from members of the cohort who did not develop breast cancer during the follow-up period.
Results In premenopausal women, glucose was associated with breast cancer risk: the age, BMI, and reproductive variable adjusted relative risk (RR) for the highest quartile of serum glucose versus the lowest was 2.8 [95% Confidence Interval (CI) 1.2 – 6.5], p for trend 0.02. Insulin showed a weaker association with breast cancer, the adjusted RR of the highest quartile versus the lowest was 1.7 (95% CI 0.7 – 4.1), p for trend 0.14. In postmenopausal women, none of the variables was associated with breast cancer risk.
Conclusions These results indicate that chronic alteration of glucose metabolism are related to breast cancer development in premenopausal women.